Pestic. Sci. 1996, **46**, 33–35

Preparation of (R)-2-(4-Hydroxyphenoxy) propionic Acid by Biotransformation*

Christoph Dingler, Wolfgang Ladner, Georg A. Krei, Bryan Cooper & Bernhard Hauer‡

BASF Biotechnology ZHB/D—A 30, 67056 Ludwigshafen, Germany

(Received 31 March 1995; revised version received 16 June 1995; accepted 12 September 1995)

Abstract: With *Beauveria bassiana* Lu 700 as biocatalyst an ecologically beneficial process has been developed for the production of (*R*)-2-(4-hydroxyphenoxy) propionic acid. The fungal strain used in this process, *B. bassiana* Lu 700, is also a very suitable catalyst for the selective monohydroxylation of other aromatic carboxylic acids.

Key words: *Beauveria*, monohydroxylation, (*R*)-2-(4-hydroxyphenoxy) propionic acid.

1 INTRODUCTION

(*R*)-2-(4-hydroxyphenoxy)propionic acid (HPOPS) is a valuable and frequently used intermediate for the synthesis of enantiomerically pure aryloxyphenoxypropionic acid-type herbicides. A possible route for its synthesis is the microbial hydroxylation of (*R*)-2-phenoxypropionic acid (POPS) which can be synthesised from (*S*)-2-chloropropionic acid isobutylester and phenol. This paper describes the selection and the subsequent improvement of a fungal strain able to perform this reaction regioselectively. With this strain a fermentation process for HPOPS production was established and the possibility of using our isolates as general biocatalysts to hydroxylate other substrates was evaluated. Rules for the hydroxylation of similar substrates have been established.

2 SCREENING OF MICRO-ORGANISMS FOR SPECIFIC HYDROXYLATION

Despite abundant literature[1] on specific monohydroxylation of aromatic compounds by micro-organisms, the hydroxylation of POPS to HPOPS has not been described. We therefore set up a screen to isolate a microorganism capable of specifically hydroxylating POPS to HPOPS. The chosen strain should meet the following criteria: high regioselectivity with no side products; lack of degradation of POPS and HPOPS; tolerant to substrate concentrations >5 g litre^{-1}; amenable to strain improvement and scale-up. The strains were inoculated in sterile medium A (20 ml)[2] and incubated at 30°C for seven days. Samples were taken daily and analyzed by GC. Of 1500 fungal and *Streptomyces* spp. bacterial strains from our culture collection, 100 strains belonging to the genera *Aspergillus*, *Beauveria*, *Paecilomyces*, *Scleroticum*, *Coprinus* and *Streptomyces* were identified as capable of hydroxylating POPS to HPOPS. Of these only *Aspergillus niger* van Teighgh, *Streptomyces hygroscopicus* (Jensen) Waks. & Henrici and *Beauveria bassiana* (Bals.) Vuill. gave $>98\%$ conversion of POPS (1 g litre^{-1}) within three days. *B. bassiana* Lu 4068 was chosen for further stain improvement because of its tolerance to POPS (>10 g litre^{-1}).

To isolate improved strains, spores were mutated with UV light (1500 erg, 10 s) or *N*-methyl-*N'*-nitrosoguanidine.[3] Several strategies were tested in order to select improved strains. As POPS and HPOPS are not used as the carbon source, and hydroxylation is not growth-associated, a specific selection system based on these features could not be applied. Mutants were therefore selected indirectly, first on the basis of POPS tolerance (100 g litre^{-1}) and second for productivity (Fig. 1).

Based on a paper presented at the symposium 'Chirality in crop protection chemistry' organised by the Pesticides Group of the SCI and held at 14/15 Belgrave Square, London SWIX 8PS on 7 March 1995.

‡ To whom correspondence should be addressed.

Pestic. Sci. 0031-613X/96/$09.00

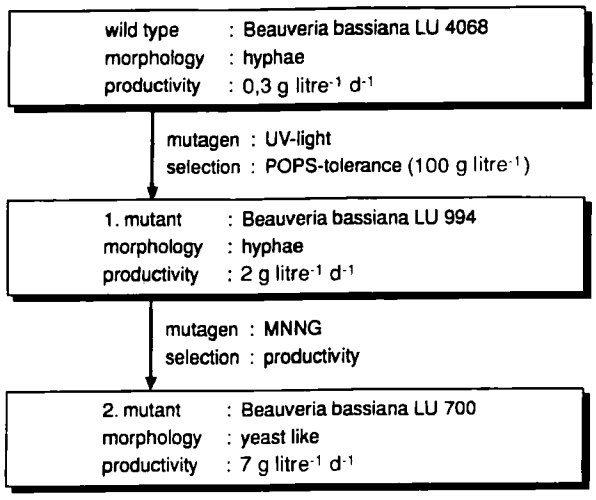

Fig. 1. Strain improvement.

The performance of the mutants with increased productivity was verified in fermentations performed on the 10-litre scale. The strains that produce mycelium were difficult to aerate adequately so that in the mutation programme we concentrated on strains which grow in a yeast-like fashion. Two mutation steps led to strain Lu 700 which had a more yeast-like morphology and a productivity of 7 g litre^{-1} day^{-1} in medium A.

3 DEVELOPMENT OF THE FERMENTATION PROCESS

Parallel to the strain improvement we started the development of the fermentation process. Special attention was paid to the nutritional demands of the strain. The composition of the trace element solution was optimized using a genetic algorithm,[4] by means of which cupric, manganese and ferric ions were found to have a great impact on productivity. By increasing the concentration of the respective ions (cupric from 0·01 to 0·75, manganese from 0·02 to 2·4 and ferric ions from 0·8 to 6 mg litre^{-1} broth) productivity was improved by 25%. Careful analysis of the physiology of the strain revealed that the process should be divided into a short growth and a prolonged production phase. This was accomplished by controlling the growth rate; thereby the overall ratio of glucose consumed per POPS molecule hydroxylated was reduced by 30%. As the total amount of glucose needed could not be added at the start, the fermentation was performed as a fed-batch process. Scale-up was performed successfully giving the same efficiency in the 100 m^3 production as in the 10-litre laboratory fermenter.

4 TESTING FOR SUBSTRATE SPECIFICITY

Having developed such a process, we were interested to know if other substrates could be hydroxylated in a similar way, and we tested the substrate specificity of the strain using more than 50 compounds. The following rules have been derived:

1. The presence of a carboxylic group and an aromatic ring system is an absolute requirement for hydroxylation (Fig. 2; **2, 3**)
2. In phenoxy-derivatives hydroxylation occurs at the *para* position if it is free (Fig. 2; **4**)
3. If more than one ring system is present, the most electron-rich is hydroxylated (Fig.2; **5, 6**)
4. If there are suitable substituents, not only ring but also side-chain hydroxylation is observed.

Where there are methyl groups in the *ortho* or *meta* position, mixed hydroxylations occur, but the presence of a *para* methyl group leads exclusively to side-chain hydroxylation. This effect is also seen for di- and tri-substituted phenoxy groups. As long as there is a *para* methyl group present, no ring hydroxylation is observed (Fig. 2; **7**), as has been verified with 2,4-dimethylphenoxy and 2,4,6-trimethoxyphenoxy substrates. The hydroxylation occurs exclusively at the methyl group in the 4-position.

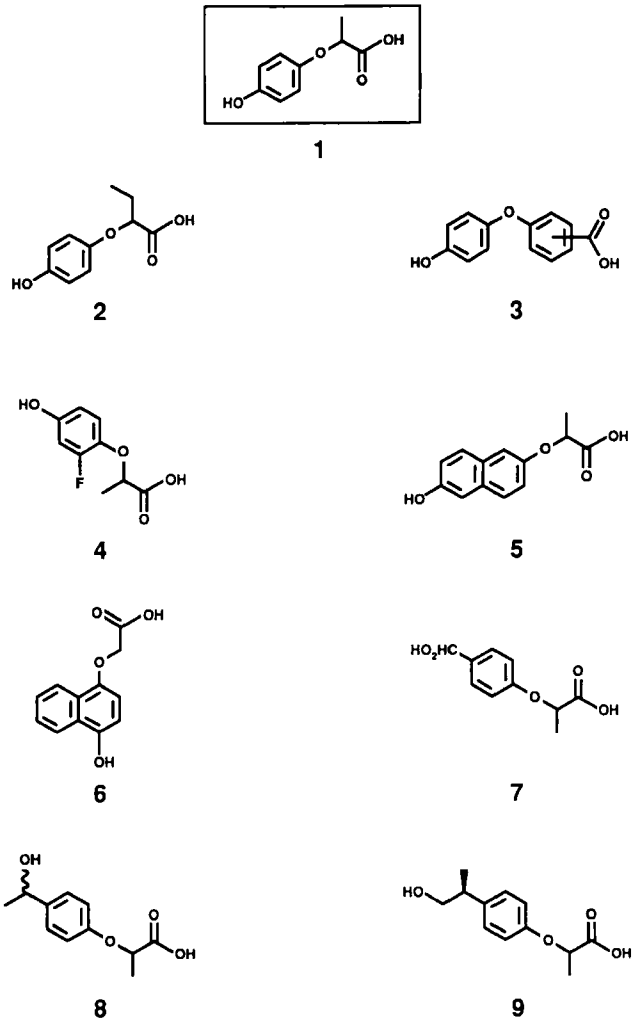

Fig. 2. Substrate specificity.

If the side chain in the *para* position is an *n*-alkyl group, the benzyl carbon is hydroxylated (Fig. 2; **8**). In such cases, no enantioselectivity is observed. However, an isopropyl group is hydroxylated enantioselectively (Fig. 2; **9**).

5 CONCLUSIONS

With *Beauveria bassiana* Lu 700 as biocatalyst we have developed an environmentally friendly process in that the fungal biomass is the only waste product that has to be disposed of. The fungus used in this process is also a very suitable catalyst for the selective monohydroxylation of other aromatic carboxylic acids.

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